## REMARKS

Claims 1-7, 22 and 24-26 are in the case.

Claims 1 and 3 are amended herewith to change "comprising passing" and "comprising separating" to –consisting essentially of passing– and –consisting essentially of separating– to exclude "treating with antibody - conjugated bead treatment" prior to screening; basis for this is submitted to be present in the application as filed at page 13, lined 25-32. Furthermore, claims 1 and 3 have been amended herewith to delete "and are free from a separating agent that comprises a ligand for isolation"; the deleted phrase was previously added to claims 1 and 3 in the response of April 12, 2004.

Claims 10 and 23 are cancelled herewith.

Claims 24-26 are new claims.

Basis for new claim 24 is submitted to be found in the application as filed at page 13, lines 25-32. The language at page 13 presents two options in the term "also possible", (1) either modifying in accordance with the language at page 13, lines 25-32 or (2) not modifying in accordance with the language at page 13, lines 25-32. Claim 24 tracks the second option. Note that negative limitations are permitted if no uncertainty or aubiguity with respect to breadth is presented, which is the case here. See MPEP Section 2173.05(I), see In re Duva, 156USPQ90 (CCPA 1967); In re Bankowski, 138 USPQ75 (CCPA 1971); and In re Barr, 170 USPQ330 (1971). Ex Parte Grasselli, 231 U.S.P.Q. 393 (Bd.App. 1983) relied on in the action of February 5, 2004 does not apply here. Rather In re Johnson, 194 U.S.P.Q. 187 (CCPA 1977) does. Johnson holds that if alternatives are positively recited in the specification they may be explicitly excluded

in the claims. That is the case here where the alternative positively recited in the application at page 13, lines 23-32, is explicitly excluded in new claim 24.

Basis for new claim 25 is submitted to be found in the application as filed on page 5, lines 21-26.

Basis for new claim 26 is submitted to be found in the application as filed at page 8, lines 9-18.

Basis for new claim 27 is found in the working examples.

We turn now to the sole objection to the specification, that is objection to reference to Tables I and II at page 40, lines 12-19 when they are omitted. Page 40, lines 12-19 are amended herewith to delete reference to Tables I and II. Reconsideration is requested.

We turn now to the objection of claim 10 under 35 U.S.C. 101. Claim 10 is deleted herewith. Reconsideration is requested.

We turn now to the rejection of claims 1-7, 10, 22 and 23 under 35 U.S.C. 112 first paragraph, as failing to comply with the written description requirement because the isolated disseminated tumor cells are denoted as being free of separating agent. The phrase "and are free from a separating agent that comprises a ligand for isolation," has been deleted by amendment herein. It is submitted that this amendment causes the rejection to be moot. Reconsideration is requested.

Claims 1-7, 10, 22 and 23 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement on the basis that the application does not enable retention on the screen of disseminated tumor cells that are "free from a

separating agent that comprises a ligand for isolation" and are essentially unchanged by the isolation process. The phrase "free from a separating agent that comprises a ligand for separation" is deleted. The fact that some aggregation clumps are retained by the sleve is irrelevant as long as not all the disseminated tumor cells are so modified; in any event, new claim 24 forecloses triggering aggregation and cluster formulation. Reconsideration of the lack of enablement rejection as set forth above, is requested.

Objection is also made to the specification not teaching of what material the screen is to be made to isolate various and different tumor cells, noting that the only working example utilizes PE material for the screen. It is submitted that there is no basis in the record for the Office Action taking a position that the screen material is important or critical. Please note that consistent with this a large number of screen materials are recited in the application as filed at page 9, line 23 to page 12, line 3. It is applicant's opinion that it is within the skill of those in the art and does not involve undue burden to select one of said materials, make a screen thereof and test whether disseminated tumor cells can be isolated with acceptable success, e.g. similar success as with the PE screen. Please note further that it can be taken from Rye et al. and Hirte et al. that in the case of nylon filters, cells do not unspecifically adhere to that material so nylon screen should work also in the instant invention. Reconsideration for the enablement rejection based on not limiting the screen material to PE, is requested. Please note also new claim 25 which defined the screen material.

Objection is made to the claims not being limited to screen of 20 µm mesh, the only mesh size in the working example.

However, it is reasonable to expect that screens having a pore width below or above 20 µm (and within the claimed range of 15 µm to 30 µm) will work as well, although less effectively. Since the method of the present invention is based on the discovery that disseminated cancer cells in blood or bone marrow have a larger size

than non-cancer cells in blood or bone marrow, two opposite effects have to be considered. On the one hand, the larger the diameter of the pores, the less disseminated cancer cells will be retained on the screen. On the other hand, the smaller the diameter of the pores, the more non-cancer cells will be retained on the screen and thus reduce the cancer cell proportion in the isolated cell fraction. When the invention was made, the applicants carried out experiments using screens with pore widths in addition to 20 µm. Briefly, mononuclear cells were obtained from blood samples of a number of cancer patients by density gradient centrifugation and then subjected to the screening process in accordance with the method of the present invention. Screens with a pore width of 200 µm, 115 µm, 74 µm, 51 µm, 38 µm, 27 µm, and 20 µm, were used. The filtrate which passed the screen with a pore width of 20µm and all retentates obtained were assessed for various RNAs and DNAs, for instance CK20-mRNA expression which is a widely accepted marker for blood borne cancer cells. The applicants have, for instance, found the following CK20-mRNA expression: (the lower the value, the higher the content of CK20-mRNA positive cells; "-" means no significant CK20-mRNA expression):

| Pore Size (µm) | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 | Patient 7 |
|---|---|---|---|---|---|---|---|
| 200 | - | - | 36.790 | - | - | - | 42.526 |
| 115 | - | - | - | - | - | - | - |
| 74 | - | - | - | - | - | - | - |
| 51 | - | - | - | - | - | - | - |
| 38 | - | - | - | - | - | - | - |
| 27 | - | - | 48.214 | 36.905 | - | 41.600 | - |
| 20 | 44.103 | 18.588 | 31.476 | 35.982 | - | - | - |
| Filtrate | 39.020 | - | 38.831 | 38.270 | 36.083 | - | - |

As can be seen from the above table, CK20-mRNA positive cells are retained on screens having relatively small pores of 27 µm and lower. Moreover, within this lower range cells can often be further fractionated into those which are retained on a screen having pores of 27 µm, those which are retained on a screen having pores of 20 µm (but pass the screen having pores of 27 µm) and/or those which may be retained on a screen having pores of less than 20 µm (but pass the screen having pores of 20 µm). This means that using a screen having pores of 27 µm allows isolation of CK20-mRNA positive cells in certain cases (here patients 3, 4 and 6) but fails in other cases (here patients 1, 2 and 5). On the other, using a screen of 20 µm is more effective in yielding CK20-mRNA positive cells (here in addition to patients 3, 4, 6, 7 also in patients 1 and 2). Merely the CK20-positive cancer cells of patient 5 pass this screen. Further, the CK20-positive cancer cells of patient 5 may possibly be retained with a screen having pores in the range of 15 µm (the lower limit of the claimed range) to 20 µm. However, this has not been tested. Moreover, with screens having pores of less than 15 µm the problem arises that more non-cancer cells will be retained on the screen so that the degree of cancer cell enrichment decreases with decreasing pore diameter. In light of these experiments it is applicant's position that the claimed method is enabled over the whole range claimed for mesh or pore width. Please note also new claim 26. Reconsideration of the enablement rejection is repeat to 15 µm to 30 µm mesh size/pore width is requested.

At page 12, the Office Action takes the position that page 41, lines 11 and 12 of the application as filed where isolated breast cancer cells that did not proliferate, suggests that the cells were altered in the isolation process. The conclusion drawn in the Office Action is not warranted. The results can beneficially indicate either that non-altered disseminated cells proliferate or not proliferate. In the case of Example 5, it should be noted that the patient had been treated with radiation and chemotherapy which may be the reason why the isolated cancer cells were unable to proliferate. So,

if the results indicate no proliferation, this reflects the state the cancer cells had *in vivo* before their isolation. Withdrawal of the lack of enablement rejection is requested.

Claim 10 is rejected on the basis that it does not particularly point out and claim the invention. Claim 10 is canceled herewith Reconsideration is requested.

Before turning to the prior art rejections, the following is presented for consideration.

The molecular characterization of disseminated tumor cells provides more information about metstatic potential, drug sensitivity and the development of therapy resistance than the analysis of the primary tumor and helps us identify subgroups of patients who might receive benefit from a particular therapy. Additionally, after removal of the primary tumor, the disseminated tumor cells and metastases derived thereof represent the therapeutic target. The emergence of new drugs directed against specific cancer related molecules makes it mandatory to stratify treatment accordingly to properly identified molecular targets, thus opening further applications for the characterization of disseminated tumor cells.

One avenue to detect and/or characterize disseminated tumor cells is based on immunological techniques, especially use of paramagnetic beads conjugated to monoclonal antibodies to capture disseminated cells by attaching to antigens expressed on disseminated tumor cell surface. Disadvantageously, in these methods, cross linking of the surface antigens can cause unpredictable effects such as apoptosis, anergy, activation and other changes in the state of the cells. Such effects can drastically change what is determined on subsequent characterization of isolated disseminated tumor cells. For example, the expression profile of a cell may be affected within a few minutes. It is impossible in such cases to rule out that the analysis data obtained in this way reflect apparent properties which the disseminated cancer cells in the body fluid did

not have prior to their isolation. As a further disadvantage, the adhering antibodies can be removed only with unfavorable consequences for the cell or not at all. If the antibodies are directed against intracellular components, fixation and perforation of the cell are necessary resulting in cell death. In these circumstances, bioassays involving living and proliferative cells, are very difficult or even impossible. A further disadvantage of purification via antibodies is cross-reactively of particular epitopes, so that normal cells may also be isolated. In added cluster formulation with blood components, e.g. platelets, and the like, may obscure epitopes important in the isolation procedure at least partially. See the application as filed at page 4, line 21 to page 5, line 12. Thus use of antigen-specific immunoadsorption as a separating technique for disseminated tumor cells prior to filtering affects the basic and novel characteristics of the product obtained.

We turn now to the prior art rejections.

Claims 1-4, 6, 7, 10, 22 and 23 are rejected under 35 U.S.C. 102(b) as being anticipated by Rye et al., American Journal of Pathology 130, 99-106(1997). Reconsideration is requested.

It is submitted that the claims distinguish Rye et al. because of the amendment thereof to recite "consisting essentially of" which excludes subject matter which would affect the basic and novel characteristics of the product obtained.

Rye et al. describes a method for isolating disseminated cancer cells, which mandatorily requires the use of antibody - conjugated magnetic beads prior to a filtration step that retains cells attached to immunobeads on a filter (immunobead filtration).

As indicted above and in the application as filed at page 4, line 21 to page 5, line 12, the immunobead filtration of Rye can provide an altered disseminated cell product.

Thus the Rye et al. immunobead filtration can affect the novel and basic characteristics of the product obtained. Since the point of the invention is to obtain a reading on metastatic potential in the body and a product that will allow decision on treatment need, the Rye et al. method cannot be relied on for this purpose. Since the Rye et al. procedure affects the novel and basic character of the result, there is no anticipation by Rye because of the consisting essentially of language inserted into claims 1 and 3, which excludes this. If in some cases in the procedure of Rye et al. there is a probability that there may be no alteration in cells isolated, there is still no anticipation. Anticipation may not be established by probabilities. See In re Oelrich, 212 USPQ 323, 326 (CCPA 1981); Hansgirg v. Kemmer, 40 USPQ 665, 667 (CCPA 1939); and Continental Can Co. USA Inc. v. Monsanto Co., 20 USPQ 2d. 1746-1749 (Fed. Cir. 1991).

The Office Action states that in Rye et al., the isolated disseminated cells proliferated (relying on page 101, paragraph bridging columns 1 and 2). However, as indicated above, the proliferation, does not mean that the cells haven't been altered, thus negating any benefit from the result.

While the present application refers to modifying the cancer cells prior to screening, it is clear from the rest of the application, that this is directed only to cases where the result would allow diagnosis of the need for chemotherapy or what that chemotherapy might be and to allow for cases where "suitable antibodies" (emphasis supplied) are used in a way that does not detract from the benefit of the results. That this is clearly not the Rye et al. method is indicated in the application:

In this regard please note page 6, lines 2-5 of the application where it is stated that an object of the invention is

> "to provide a mild method for isolating cancer cells from cell-containing body fluids which has no or only a negligible effect on the state of said cancer cells" (page 6, lines 2-5)

Note also that after a description of the disadvantages of antigen specific immunoadsorption (includes the Rye et al. method) in the application as filed at page 4, line 21 to page 5, line 8), it is pointed out, to overcome such disadvantages, that

> "the cancer cells ought to be isolated from the body of fluid essentially unaltered, i.e. not attached to constructs due to the isolation procedure, such as glass beads" (page 5, lines 21-24).

From the above, it is submitted that the Rye et al. method is required to be excluded from the claims to assure the beneficial results thereof. It is submitted that the insertion of "consisting essentially of" into the claims, effects this.

See also new claim 24 which explicitly excludes the Rye et al. process. See also new claim 27, where what is screened is positively defined in terms where there can be no immunobead filtration.

Reconsideration and withdrawal of the rejection based on Rye et al. American Journal of pathology, is hereby required.

Claims 1-4, 6, 7, 10, 22 and 23 are rejected under 35 U.S.C. 102(e) as being anticipated by Fodstad, Hoifodt and Rye U.S. Patent No. 6,265,229. All three co-inventors are co-authors of the Rye et al. article used as a basis of the rejection stated above. Here again, as in the Rye et al. article a method for isolating disseminated cancer cells is described, which mandatorily requires the use of antibody conjugated

magnetic beads prior to a filtration step that retains cells attached to immobeads on a filter (immunobead filtration). Reconsideration is requested. It is submitted that U.S. Patent No. 6,265,299, is defective for anticipation purposes for the reasons given above in distinguishing Rye et al. American Journal or Pathology.

Claims 1, 3, 4 and 5 are rejected as being unpatentable over Rye et al., American Journal of Pathology 130, 99-106 (1997) or Fodstad, Hoifodt and Rye U.S. Patent No. 6,265,229 (hereinafter both together are referred to as Rye) in view of Hirte et al. Gynelogic Oncology 44, 223-226 (1992). Reconsideration is requested.

It is noted that the claims are limited to utilizing a body fluid selected from the group consisting of blood and bone marrow.

Rye discloses isolating tumor cells from blood, bone marrow, ascitic/pleural fluids and enzyme-digested tissue biopsies. Since Rye mandatorily requires use of anitbody-conjugated magnetic beads prior to a filtration step and the claims exclude these and Rye does not indicate any benefit when the beads are excluded, relevance has to be placed on combination with Hirte for the obviousness rejection to demonstrate that beneficial results can be obtained when omitting the anitbody-conjugated magnetic beads of Rye. However, Hirte is limited to harvesting tumor cells from ascitic fluid so Hirte cannot make it obvious that beneficial results can be obtained on excluding the conjugated magnetic beads of Rye to obtain tumor cells from the body fluids to which the claims here are limited, i.e. blood and bone marrow, rather than ascitic fluids.

The case for ascitic fluids is much different from the cases for blood and bone marrow.

Ascitic fluid naturally contains a high proportion of tumor cells. For instance, in Hirte et al. it is said that cell suspensions obtained from ascites consisted of a mixture

of 60 to 80 % tumor cells mixed with non-malignant cells (Hirte et al., page 223, right column, last sentence under item 1). In contrast thereto, disseminated cancer cells which circulate in blood or bone marrow represent only a very minor portion of the cellular contents. In blood the ratio of disseminated cancer cells to mononuclear cells is usually about $1:10^6$ or less. In bone marrow, said ratio sometimes is 5- to 10-times higher but nevertheless very low as compared to the ratios found in ascitic fluid.

Moreover, there is a difference in terms of how said tumor cells have detached from the primary tumor. While the tumor cells present in ascitic fluid are shed from the solid tumor, dissemination into blood and bone marrow is based on an active hematogenic process. Said hematogenic process requires that the primary tumor is vascularized and that certain tumor cells have acquired the capacity of entering into the blood vessels. This means that the hematogenic process provides a kind of clonal selection of specialized tumor cells while shedding represents a mere mechanical detachment of primary tumor tissue. Accordingly, Hirte et al. describe that the tumor cells were isolated as multicellular aggregates (cell clusters) and as large cell clumps.

It is submitted that these fundamental differences would have prevented the skilled person from envisaging the use of a screen for isolating disseminated cancer cells from blood or bone marrow despite Rye and Hirte et al.

Furthermore, applicants have data which shows that the disseminated cancer cells which are isolated in accordance with the invention (from blood and bone marrow) correlated with the clinical outcome of the patients whereas cells isolated by immunomagnetic separation did not correlate with a higher probability of disease recurrence.

In particular, disseminated cancer cells from blood samples from breast cancer patients were isolated by filtration without immunomagnetic separation (hereinafter

referred to as M1 cells) and disseminated cancer cells from blood samples from breast cancer patients isolated by immunomagnetic separation (hereinafter referred to as M0 cells). In both cases the cells could be identified as cancer cells due to the presence of genomic imbalances (DNA mutations, amplifications and losses of heterozygosity). The prognostic meaning of the M1 cells and M0 cells were compared. The results are shown in Figure I attached. As shown in Figure I, in the M0 cells an increased number of genomic imbalances did not correlate with a higher probability of disease recurrence whereas in M1 cells an increased number of genomic imbalances did in fact correlate with a higher probability of disease recurrence. Thus, M0 cells do not have the prognostic value of the M1 cells. Comparison of immunomagnetic separation and filtration separation is set forth in the following post-published articles where inventor Giesing was a co-author, namely (1) Uciechowski, P., et al., British Journal of Cancer 83(2), 1664-1673 (2000), (2) Bockmann, N. et al., Biomolecular Engineering (2001) 95-111, and (3) Giesing, M, et al., The International Journal of Biology Markers 15(1), 94-99(2000). Copies of the three articles are enclosed. These articles indicate (i) that disseminated cancer cells are separated by the claimed invention; (ii) that the disseminated cancer cells, when isolated in accordance with the present invention, do not reflect the genotype of the primary tumor; and (iii) that the detection of disseminated cancer cells which are isolated in accordance with the present invention correlates with the clinical outcome of the patients.

Further, differentiation can be made with respect to tumor cells from ascitic fluid and tumor cells from blood and bone marrow. For instance, the applicants examined a woman (53 years) with a diagnosis of ovarian carcinoma and metastatic spread into the peritoneal and pleural cavities. The geno- and phenotyping of ovarian tumor tissue, tumor cells from ascitic fluid, and disseminated cancer cells isolated from blood using screening separation only, clearly revealed that tumor cells from ovarian tumor tissue and from ascitic fluid showed a similar geno- and phenotype which, in turn, is markedly different from the geno- and phenotype of the disseminated cancer cells. The data is

presented in Table I attached. As a consequence, cells from ascitic fluid would not be expected to have the prognostic meaning the disseminated cancer cells have.

Applicant's will supply this data if it is necessary to obtain allowance.

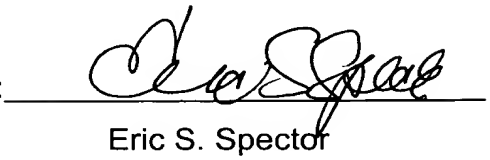
It is noted that applicants have made a remarkable and important discovery. Applicants were the first to recognize that disseminated tumor cells are a separate tumor entity, i.e. distinct from the tumor cells, that tumor cells evolve in circulation because they are in a different environment, that the disseminated evolved tumor cells are the ones involved in metastases, and that selection of treatment based on the evolved tumor cells is important to select treatment for metastatic cancer since selection of treatment of metastatic cancer based on the primary tumor cells can be ineffective or even harmful. It is clear from the application as filed that immunomagnetic separation can skew the results so that disseminated cancer cells as function in the body are not of isolated and in such case immunomagnetic separation obviously would be counterindicated. There is clearly an important novel invention here which can prove unique important results. If the PTO is unhappy with how said invention is defined, it should work with the undersigned to reach a definition that it is happy with.

Allowance is requested.

A Power of Attorney (two forms) to the undersigned and revoking prior powers, is enclosed.

The correspondence address should be changed in accordance with this and the undersigned should be contact person for the PTO.

Respectfully submitted,
BACON & THOMAS, PLLC

By: 

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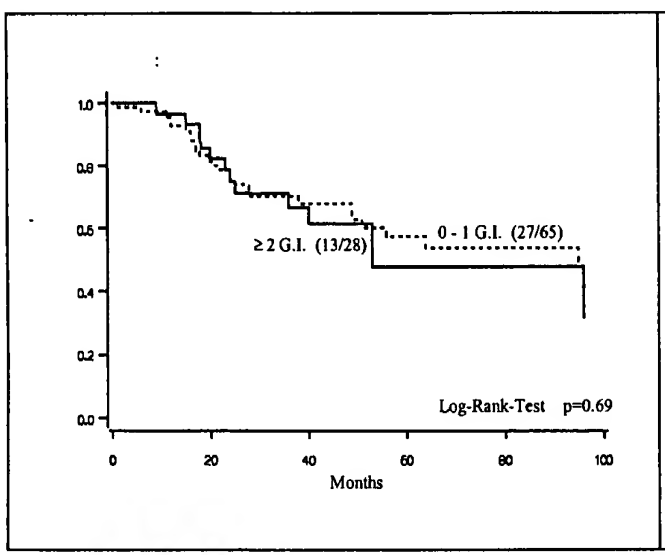


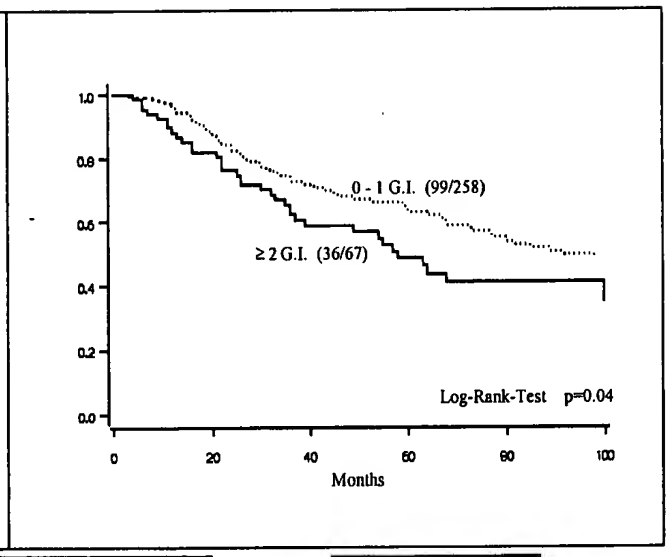


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Figure 1